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1930

SEPARATE.

FUNGI OCCURRING IN CACAO BEANS.

BY

R. H. BUNTING.

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—

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PLATES VI AND VII.

Moulds in cacao beans (drawn natural size).

FIGURES.

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(3) Aspergillus glaucus perithecial stage.

(4) Aspergillus fumigatus.

(5) Aspergillus fumigatus growth developed at 40°C.

(6) Aspergillus tamari.

(7) Aspergillus flavus, light coloured form.

(8) Aspergillus flavus, light coloured form.

(9) Aspergillus flavus, dark coloured form.

(10) Aspergillus niger.

(11) Aspergillus ochraceus.

(12) Penicillium sp. entering germinated bean.

(13) Penicillium sp.

(14) Mucor cymose species, early stage.

(15) Mucor cymose species, later stage.

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Plate VI & VII.

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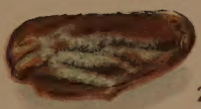
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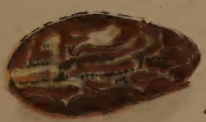
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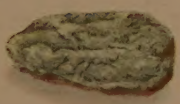
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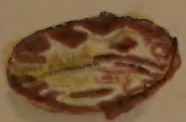
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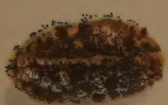
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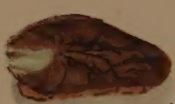
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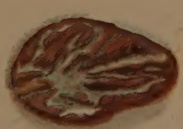
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PAPER No. VIII.

FUNGI OCCURRING IN CACAO BEANS.

By R. H. BUNTING.

One of the most frequent defects of cacao beans exported from this country is due to the presence of one or more of a number of moulds on the embryo of the prepared beans.

The presence of any internal mould deteriorates the value of cacao intended for human consumption; cocoa* and chocolate manufacturers are unable to utilise it in appreciable quantities on account of the flavour it imparts, and the markets, influenced by the pure food campaign, are becoming more resolute in refusing to purchase parcels of cacao containing more than a limited percentage of moulded beans.

The desirability of ascertaining what organisms cause moulding was influenced not so much on account of their direct effect on the constituents of the produce as because of the factors which contribute to their development, since it is their presence rather than their function which is objectionable.

In early attempts to ascertain the minimum amount of moisture that is necessary to induce or to maintain the growth of moulds in cacao beans, it soon became apparent that for this reason alone it would be necessary to separate as accurately as possible the various organisms for individual treatment. This paper is an endeavour to elucidate, morphologically rather than physiologically the ever growing number of species involved.

Considerable difficulty has, however been experienced in delimiting the bounds of variation which occurs in shape and size of almost any given part of the organisms concerned, so that one has to rely upon general characterisation rather than upon definite characters for the determination of individuals.

One or possibly more of the undetermined species may be new to science. Descriptions and illustrations of them are given and their position in nomenclature left to special systematists.

GENERAL CLASSIFICATION.

From the practical point of preparation, the moulds affecting cacao-beans may be divided into three sections according to the conditions in which they are found.

N.B.—The word "cocoa" means the manufactured, commercial powder. "Cacao" is used for any raw part of the plants.—EDITOR.

I. Apart from numerous yeasts, the fungi which are first encountered in the preparation of cacao-beans are thermophilic and, in one case, thermogenic ; they occur with some frequency in fermenting masses and may increase the temperature beyond that of an unaffected fermentation.

II. In prepared beans which have a high moisture content the most prevalent moulds are those of the larger *Aspergillus* species having secondary sterigmata.

III. The third section occur on what is usually considered as dry cacao and which has been stored for some time, and consists of a member of the *Aspergillus glaucus* group with an ascigerous stage, and a species of *Penicillium*.

As an example of the complexity of problems relating to the moulding of cacao beans, it may be mentioned here that in several instances, where indeterminate mycelia were found infesting embryos, one half of a bean incubated under aseptic conditions at 40°C. resulted in the development of one or other of the thermophilic moulds, whereas the complementary portion of the bean grown at laboratory temperature developed one or more of the other moulds.

The commonest, and therefore most important, of the cacao moulds are confined so far as observations have gone, to the orders Mucoraceae and Aspergilleae. Other fungi occasionally occur in cacao-beans, such as *Botryodiplodia theobromae* on beans inadequately prepared from diseased pods, *Cladosporium* sp. on beans stored for a long time. *Monilia sitophila* is of still more rare occurrence, whilst many searches for species of *Actinomyces* have, with one known exception, given negative results, so that Ciferri's observations regarding obnoxious fungi on cacao-beans in Dominica (*a*) would seem to be inapplicable to the produce of this country.

The genus *Aspergillus* provides the chief offenders in local cacao-beans, its species include members of the following group :—

A. fumigatus, flavus (two forms), *glaucus, ochraceus, tamari* and *niger* (three forms). Of the Mucoraceae, a cymose species of Mucor, two species of *Absidia* and one of *Circinella* have been found.

I. THERMOPHILIC MOULDS.

Ia. Aspergillus fumigatus Fresenius group.

(Plates VII, figs. 4 and 5. VIII, fig. 5, IX, fig. 4.)

Frequently found in fermenting heaps in native farms, particularly when such masses of beans are not disturbed. It occurs as a fairly compact glaucous mould approximating the colour of figure 5 on Plate VII, adherent to the surface of the

(*a*) Ciferri : Studien über Kakao I. Centralblatt, für Bakt. Ab. 2, LXXI, 1–7. pp. 80–93, 1927.

Plate VIII.

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beans, and occupying the warmer areas of the heaps. It is absent from the centre of the heap probably on account of its development being inhibited by the high saturation of CO_2 in that region since the temperature generated there never reaches the thermal death-point.

Its thermogenic activity is indicated by the fact that, in one mass where it developed, the temperature rose to 54°C., i.e., 8 degrees above the usual temperature attained in box-fermentations. Spores suspended in a liquid nutrient medium, and subjected to a temperature of 67°C. for ten minutes, survived to form abundant sporophores, and it is possible that further tests at higher temperatures may also show survival.

By colorimetric methods the cultivation of *A. fumigatus*, derived from local cacao-beans on media of the approximate pH indices 4.5, 5.6 and 7.2 produced in three days reactions which gave 6.5, 7.0 and 7.5 respectively.

The question of pathogenicity to animals of the local strain of this species arose as a result of numerous references in literature to various mycoses in man and fatal diseases in birds which are accredited to *A. fumigatus* Fres. An attempt to test whether the cacao-bean *fumigatus* was pathogenic to animals by infecting the abrased skins of a monkey and a dog gave negative results, but since animal diseases caused by members of this group are usually pulmonary, or such as affect the mucous membranes, the trial should not be regarded as conclusive.

Thom and Church in their monograph of the Aspergilli differentiate, in their discussion of the group of which *A. fumigatus* Fres. is the type, between perithecial forms and those with similar conidial forms but no perithecia [(*b*) pp. 219]. Neither in cacao-beans nor on artificial media has the Gold Coast strain been found to produce perithecia. It falls naturally within the general characterisation of the conidial stage of the group as given by these authors. Morphologically therefore, it may safely be regarded as *A. fumigatus* Fres.

Conidial heads of this mould taken from a cacao-bean are illustrated at a magnification of approximately 32 times in fig. 5 of Plate VIII, and its general appearance within a cacao-bean is shown on Plate VII by fig. 4 ; figure 5 of the same Plate shows the growth which developed at a temperature of 40°C. Colonies both on the testae and embryos of cacao-beans, and on Dox's agar medium* are velvety, rarely if ever approaching

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b) Thom and Church : The Aspergilli, 1926.

* Dox Agar, as used for the moulds mentioned in this bulletin :—

Pot : nitrate	2.0	Cane-sugar	15.0
Mag. sulphate	0.5	Ferric chloride	1 drop.
		Agar. ..	15.0
Pot. phosphate	1.0	Water up to	1,000.0

a floccose condition, in colour they vary according to age from glaucous green (Ridgway XXXIII, 39″, d) in young cultures and in cacao-beans to olive and later to natal brown (R. XL, 13‴, m).

The sporophores (Plate IX. fig. 4) are small and densely crowded, they are smooth and unseptate, hyaline at first becoming more or less fuliginous in old cultures, 130 to 400μ in length widening gradually from 3 to 5μ at the base to 6 to 7μ below the vesicle, which is elliptical, sometimes with a depressed apex, and up to 24μ in diameter. The sterigmata are in a single series crowded around the upper part of the vesicle and parallel to the axis of the conidiophore, they measure 6 to 10 by 2.5μ. Conidia are borne usually in cylindrical heads up to 150μ long by about 30μ in diameter, but in all cultures some more or less loose, globular heads may be found; the spores are spherical, 2.5 to 3μ in diameter, tending towards an oval, less regular and slightly larger form in old cultures up to 4 x 2.5μ; in a mass they are green in colour.

The smaller and smooth spores, non-septate sporophores and cylindrical heads distinguish this species from *A. glaucus*—the only other form with which it is likely to be confused on cacao-beans. The facts that the latter fungus produces a perithecial stage, cannot be regarded as thermophilic, and utilises a drier substratum, are also of diagnostic assistance.

The importance of *A. fumigatus* in problems relating to the moulding of cacao-beans lies less in its persistence as an active mould in the dry produce, than in the fact that it occurs in fermenting masses, and may be the means by which other and more persistent moulds enter through the protecting seed-coat. Experimental work on this point has shown that the fungus can penetrate the testa of a fermented bean, as was to be expected from the fact that it has been recorded (Thom and Church; pp. 67 and 68) that certain members of this group have been shown to be capable of destroying cellulose, and to be active in the destruction of the pentosans of maize-forage and rye-straw (*c*).

Ib. Mucor sp.*

(Plates VII, figs. 14 and 15, VIII. fig. 10, XI).

The second thermophile found in fermenting heaps belongs to the cymose group of the Mucors, and is probably a new species. It develops from a white floccose vegetative mycelium to a somewhat more compact, sporangial mycelium of a deep olive grey colour (R. LI. 23‴″), which extends to a depth of three inches from the surface of the heap. It is frequently associated with *Aspergillus fumigatus*, and is probably more common in native fermenting heaps than the latter.

(*c*) Schmidt, Peterson and Fred: Soil Science XV. pp. 479–488; 1923.

* described by Ralf A. Leudner as Mucor bainieri n. sp. in Bull Soc. de Gaieve 2nd Ser. pp. 256-263. 1930.

IX

This Mucor has also been cultivated from cacao-beans prepared for export, and has been found on copra stored locally. Early stages of its development in the interstices between the folds of the cotyledons of a cacao-bean are of a light grey colour, which changes to a light olive buff, such as are shown in figures 14 and 15 of Plate VII.

A suspension of spores in a liquid medium survived an exposure to a temperature of 67°C. for ten minutes. The association of this fungus with *Aspergillus fumigatus*, and the fact of its thermophilic tendency, permit the suspicion that it may be parasitic on warm blooded animals as is *Absidia lichtheimi* (*Mucor corymbifer* Cohn.) a species for which it was at first mistaken.

Prof. S. F. Ashby (in lit.) stated that he was unable to find a place for this *Mucor* in any of the recognised species of the cymo-Mucors where it obviously belongs, that its action on starch is weak, and that he could detect no alcohol in cultures on malt extract.*

The fungus is shown (x 32) in fig. 10 of Plate VIII as a group of sporangia from a young colony; details of its structure reproduced from camera lucida drawings are given on Plate XI.

The mycelium is hyaline, but becomes dark brown in old colonies, it shows a decided tendency toward a torulose condition with occasional intercalary chlamydospores which are from 11 to 30μ in diameter. Sporangiophores are hyaline, soon becoming dark brown, they arise from a narrow base which may be not more than 3μ in width; on some media sporangiophores and pedicels develop a coarse obese appearance than can only be regarded as abnormal. The sporangia are borne in simple or compound cymes, an arrangement which is sometimes confused by the development of a number of short pedicels arising from the main axis near the terminal sporangium. The pedicels usually increase in width from a narrow base up to 15μ at the insertion of the sporangium, they become septate below the terminal sporangia, and are fuliginous above, and brown below the septum.

The sporangia are spherical, erect, minutely echinulated with crystals, and diffluent; they vary in size up to 80μ in diameter, the smaller ones sometimes being almost apophysate.

*At the time of going to press Professor Ashby (in lit.) states that Professor Lendner confirms the suspicion that this is a new species, and will describe it.

The columellæ are faintly fuliginous, either smooth or rough, subspherical to oval, up to 50μ in diameter. The spores are also somewhat fuliginous, subspherical to oval, frequently irregular in shape, smooth, differing greatly in size usually 3-5μ in diameter, but up to 8.5μ.

II. HYGROPHILIC MOULDS.

The four groups of *Aspergillus* which are represented in cacao-beans with an abnormally high moisture content are differentiated chiefly by colour into *Aspergillus niger, tamari, flavus* and *ochraceus*. Their presence may be regarded as indicating either that the produce has not been dried sufficiently during preparation, or that it has come into contact with moisture subsequently. For this reason they are not considered of such economic importance as are those which infect drier beans.

Ia. Aspergillus niger Van Tieg. group.

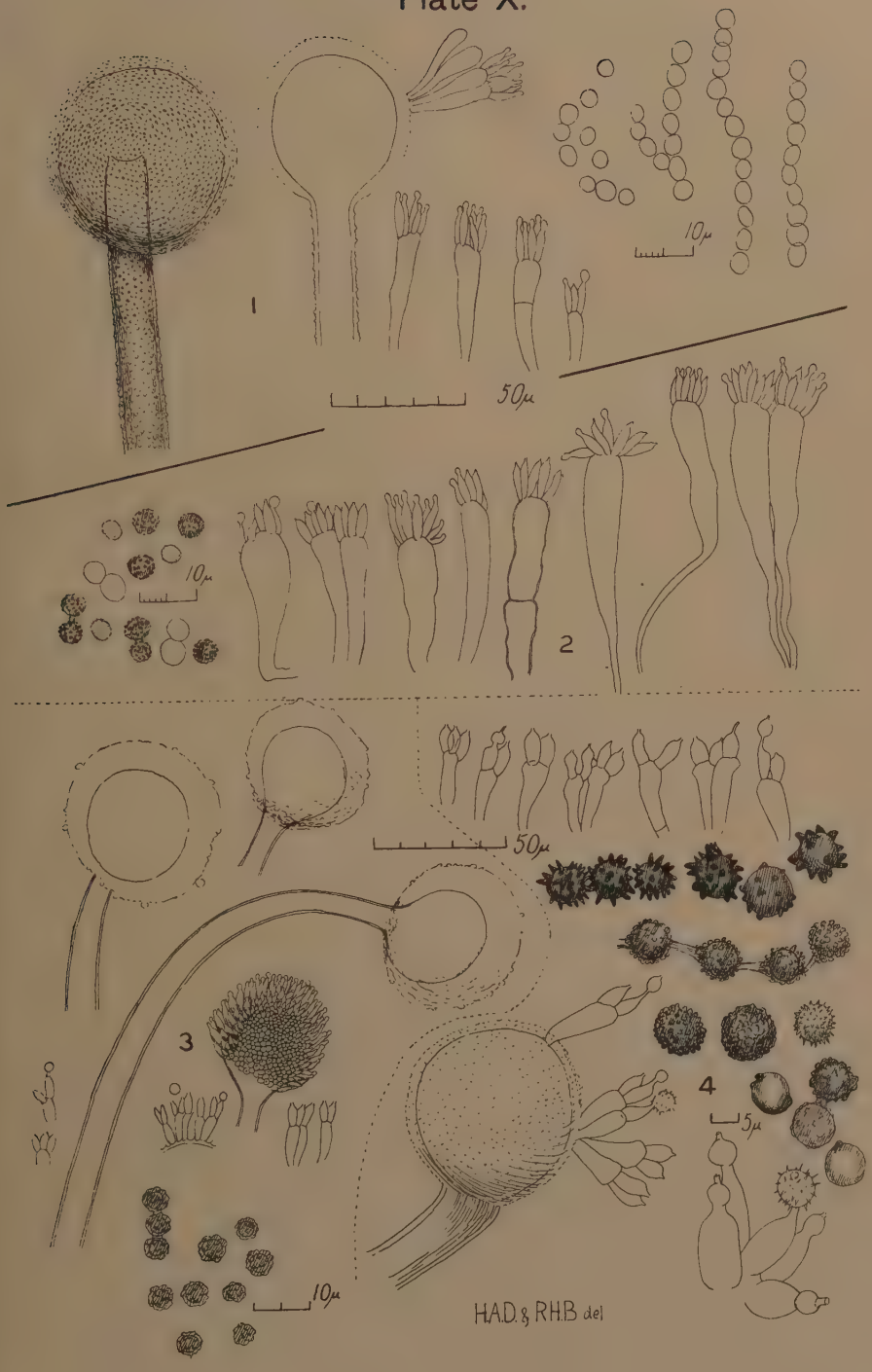
(Plates VII, fig. 10, VIII, fig. 7, X, figs. 2, 3, and 4).

There are at least three morphologically distinct forms of *A. niger* found in connection with cacao-beans in this country. They are shown grouped together in fig. 7 of Plate VIII at which magnification they cannot be readily distinguished from one another, since each may present a warm deep brown to almost black, soft, mealy appearance when growth is luxuriant. The conidial heads, of which the mass is composed, vary in diameter at maturity from 120μ to more than a millimetre; at younger stages they are usually hyaline and may pass gradually through darkening olivaceous tones, or may with few perceptible changes of colour reach the dark brown colour of maturity; they are always spherical at first, but may split up into columnar segments, remain compact, or become shaggy. The vesicles are always spherical, differing very considerably in size, usually thick walled; and either smooth or punctate at the bases of the sterigmata which arise from their entire surface. They, like the stalks of the sporophores, are usually coloured.

The three local forms are detailed on Plate X under the figures 3, 4 and 2, which for convenience of description may be cited as A, B and C respectively.

Form A. (fig. 3) appears to conform to A. *luteo-niger* (Lutz.) which was collected on fermenting cacao beans in San Thome (Thom and Church; p. 166). In young growths conidia are borne directly on the primary sterigmata which are about 10μ in length; more typically one to three secondary sterigmata are borne on those of the primary series which may then attain a length of 30 or even 40μ. The thick walled, dark-

Plate X.



brown, tuberculate, globose spores of 3.5–5μ diameter are, in conjunction with the comparatively short primary sterigmata, the chief characteristics of this form. It should also be noted that usually the stalk of the conidiophore decreases in width toward its junction with the vesicle.

Form B. (fig. 4) differs from A. chiefly in the size of the spores which are from 7 to 12μ in diameter. They are globose, fuscous to dark-brown, occasionally almost smooth, spinous, tuberculate or with numerous conical protuberances that are up to 3.5μ in length. The primary sterigmata are usually truncate and of rather more generous proportions than those of form A., they bear from one to three secondary sterigmata. The sporophore stalk shows no tendency toward a narrow neck, but is frequently roughened, apparently by pits.

Form C. (fig. 2) is distinguished from either of the other forms by its elongated primary sterigmata bearing up to twelve or possibly more, secondary sterigmata. Those of the primary series are clavate, arising from a narrow base sometimes not more than 1μ in width, they vary in length from 40 to 110μ, and are occasionally septate. The conidia are spherical, 4–5μ in diameter and are comparable with those of form A. except that a larger proportion of light coloured, smooth, or almost smooth, spores usually occur. The sporophores are usually smooth, hyaline or coloured, up to 24μ in width, and split readily under pressure into strands, they arise from a slender base frequently involved in a tangle of narrow, thin walled hyphae that make it difficult to discern the foot-cell.

IIb. Aspergillus tamari Kita group.

Plates VII, fiig. 6, VIII fig 3, IX, fig. 6).

In cacao beans this mould presents the white to medal-bronze (R. IV. 19′, m) colour shown by fig. 6 of Plate VII, and darkens to mummy-brown (R. XV. 17′.m) in old cultures; grown on Arbutin agar it became a deep beetroot colour. Its enzymatic activity has been found to be positive in the case of tests for amylase, emulsin, lipase, protease, erepsin, trypsin and amidase, and it is reported as apparently not producing acid.

A. tamari is not found with great frequency in cacao beans; it occurs as conspicuous masses of small and compact, or much larger, shaggy, globose spore heads, that may usually be distinguished from a similarly coloured strain of *A. flavus* by their greater length of stalk. The sporophores are usually long, hyaline, sometimes pitted, and arise from a slender base widening gradually towards the vesicle. The vesicle is globose and thin walled bearing sterigmata on the whole of its surface;

in some cases simple sterigmata bearing chains of conidia occur around the base of the vesicle. Commonly there are two series of sterigmata, the primary ones being 10–20 x 6-10μ in size, compactly arranged and bearing rarely more than two secondary sterigmata which may be of equal or greater length. The conidia are almost pyriform to obovate, yellow brown in colour, tuberculate when fully formed, 7–10 x 6–7μ. Its characteristic features other than colour are the usually equal length of secondary and primary sterigmata and large spores.

IIc. Aspergillus flavus group.

(Plates VII, figs. 7, 8 and 9, VIII figs. 1 and 2, IX, fig. 5).

As in the case of local copra moulds, there appear to be two forms of the *flavus* group occurring on and in cacao-beans. The commoner is represented by figure 8 of Plate VII and the other by fig. 9 of that Plate. Figures 1 and 2 of Plate VIII indicate the distinctive colours of the two forms at the magnification given.

In culture the colonies are widespread and smooth, zonate when grown on maize meal agar ; those of the lighter coloured form varying between parrot green (R. VI. 31. k) to warbler green (R. IV. 23. k) and darkening with age to bistre (R. XXIX. 15″. m). The darker coloured form is medal bronze (R. IV. 19. m) with a border of pyrite yellow (R. IV. 23. i) on Dox agar. The mycelium and young sporophores are hyaline, and as the fungus grows rapidly an early growth completely filling the interstices of a cacao-bean may appear white, as does the advancing periphery of a developing colony on artificial media.

There are no very individualistic anatomical characters differentiating this group from others of the larger Aspergilli. The hyaline, minutely pitted and occasionally septate stalks of the sporophores increase gradually in width from a narrow base to their junction with the elliptic or globose, yellow-brown, punctate, vesicles. There are typically two series of sterigmata but heads with a single series of short sterigmata are common in the lighter coloured form. In this strain columnar heads of spores are not uncommon owing to the development of sterigmata being sometimes limited to the upper two-thirds or smaller proportion of the vesicle. The spherical to somewhat pyriform conidia of the two forms differ ; those of the lighter colour being smaller—3.5 to 5.5μ in diameter and either almost uncoloured or yellowish with indistinct markings, whilst those

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up to 50μ in length, and bear from 3 to 6 secondary sterigmata 10-12μ long. The chains of conidia are sometimes branched and consist of almost smooth or unconspicuously roughened' globose to elliptical spores from 3 to 4μ in diameter along their axis. Sclerotia are produced abundantly and average 500μ in diameter.

In addition to the Aspergilli, there are at least three species of the Mucoraceae found in or on wet cacao-beans in this country.

IIe. Circinella spinosa Van Tieg. et Le Monn.

(Plates VII, fig. 17, VIII, fig. 9, XIII, fig. 2).

A few samples of mouldy beans which had been purchased by small buyers were found to be infected by a grey brown mould which developed rapidly in a damp chamber covering the surface of the beans, and filling the interstices between the cotyledons. On Dox agar the mould, at first white, grew luxuriantly filling the tube with a floccose, light brown olive (R.XXX.19″.k.) growth of sporangiophores.

The sporangiophores consist of sympodia of circinnate pedicels bearing spherical sporangia.

The sporangiophores vary considerably in width (7-16 μ) and are indefinite in length, as may be seen by figure 2 on Plate XIII. They arise from a sturdy mycelium which bears numerous delicate rhizoid branches (fig 4). The sporangiophores are at first hyaline but in a few weeks they assume a cinnamon buff colour (R.XXIX.17″.b.) and may be incrusted with minute crystals.

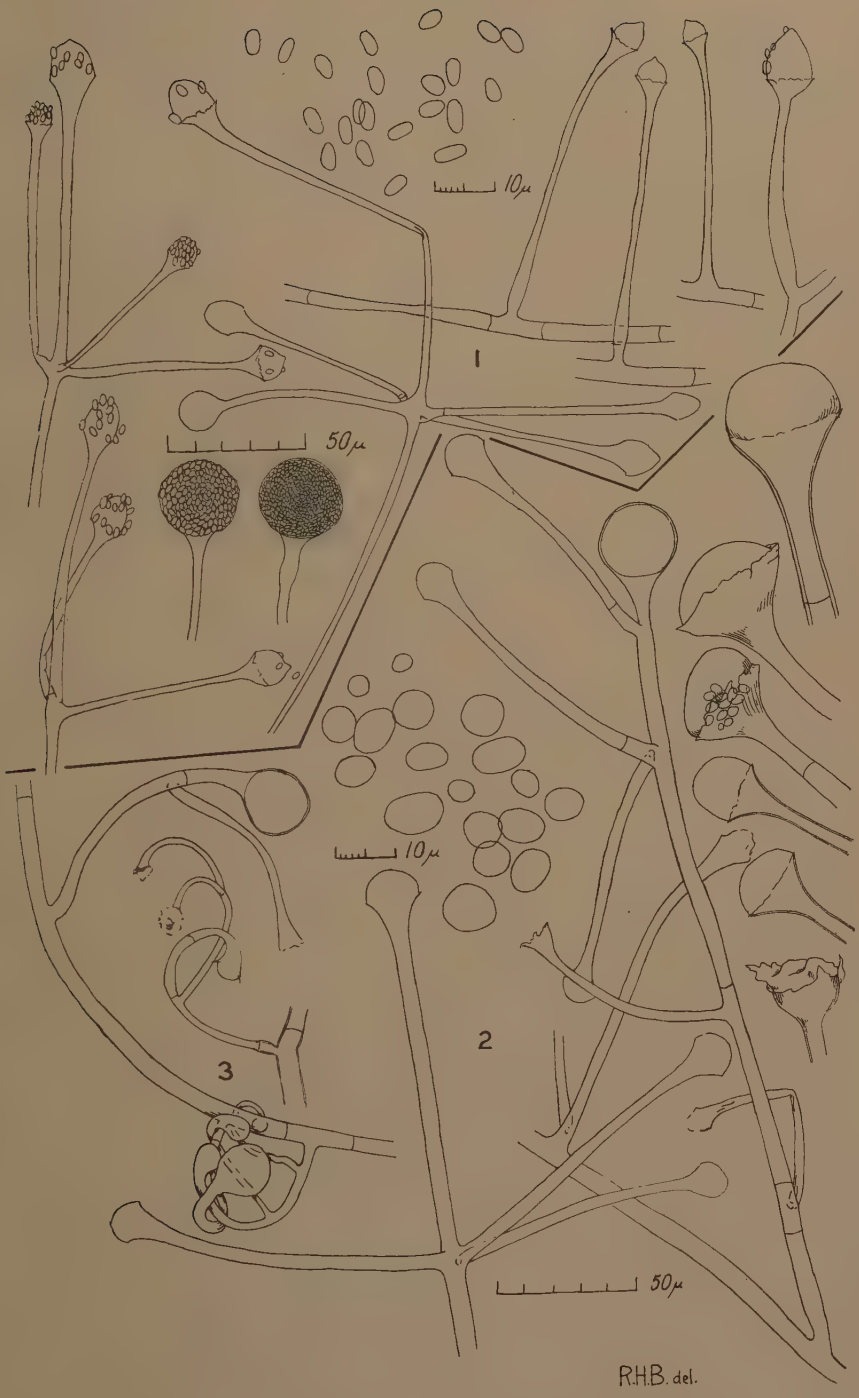
The pedicels are always recurved at the upper end; a septum is formed below the recurved portion, and below the septum the next pedicel is developed. The system of branching is thus usually a simple one. Sporangia are spherical, hyaline cream or clay colour (R.XXIX.17″.), they have persistent walls and measure from 30 to 90 μ in diameter. The columella is variable in shape from cylindrical to pyriform, sometimes almost spherical, hyaline to buff, and 25–80 X 12–57μ in size. The spores are hyaline smooth, spherical or subglobose 3-6μ in diameter.

Zygospores have not been found.

Cultures of the fungus were sent by the Imperial Bureau of Mycology to Professor Lendner, who kindly provided the definite diagnosis.

(*C. spinosa* is found in Europe on the excrement of man, horses and rats.)

XII



IIf. Absidia regneri (Lucet et Const.) Lendner.

(Plates VII, fig. 16, VIII, fig. 8, XII, fig. 2),

A white mould which has been found once or twice on beans prepared for export occurs as both an external and internal infection. It develops rapidly at a temperature of 40°C. and was originally found in beans which contained 24 per cent. of moisture. In culture the growth produced was floccose, white and abundant, on cacao-beans the mould appeared to be slightly coloured.

The sporangiophores are slender, not exceeding 12μ in width, they tend to become tinted and, on some media, are minutely echinulate at the apical end. The method of branching is diverse ; a main axis, terminating in a sporangium, may produce single erect pedicels below a septum, short apparently hypertrophied sympodia of circinnate pedicels, or a verticillate arrangement of straight pedicels. The pedicels are usually more slender than the axis on which they are borne, and are somtimes septate; they enlarge at their apices into a funnel or cup-shaped apophysis which supports the spherical sporangium. The sporangia are colourless and usually completely diffluent, those illustrated by fig. 8 of Plate VIII appeared dark at the magnification at which they were drawn possibly owing to the inclusion of air. The columella is smooth, delicate, sometimes collapsing into the apophysis, oval in vertical section and usually depressed, its height never exceeding its width. The spores are hyaline, oblong to irregularly spherical, smooth, measuring 3–9 X 2.5–5μ, or from 3–7.5μ in diameter when nearly spherical.

Zygospores have not been found.

The interest of this fungus lies in its thermophilic tendency as a possible invader of fermenting heaps.

Professor Lendner, in correspondence with the Imperial Bureau of Mycology, advises the adoption of the binomial *A. regneri* for this fungus. He states (" Les Mucorinées de la Suisse, " 1908) that *A. regneri* does not grow at a temperature of 51–52°C, but that the nearly related *A. truchisi* (Lucet et Const.) does grow at that temperature. Both are pathogenic to the rabbit.

IIg. Absidia capillata Van Tieg.

(Plate XII, fig. 1).

A second species of the genus Absidia has been found on the surface of fermented cacao-beans during the early stages of the drying process. The fact that the infected beans contained 38. 5 per cent. of moisture probably accounts for the fungus not being known to occur within dry produce.

This species of *Absidia* differs from the preceding one by its more slender proportions, its sporangiophores rarely exceed 7μ in width, its spores are also smaller and more uniformly oblong in shape, and the height of its columella is equal to, or frequently exceeds the width.

It produces a white, floccose, growth in culture which rapidly fills the tube with an aerial fertile mycelium. The sporangiophore branches apically with a verticillate arrangement of long fragile, straight pedicels; simple pedicels may also arise below a septum on the main axis, and are sometimes slightly swollen at or near their junction with the sporangiophore. The sporangia are colourless spherical and diffluent, 16 to 30μ in diameter. The columella is typically conical, sometimes mammiform, and occasionally oval. The spores are oblong, hyaline and smooth, 4.5–5.5 x 2–3μ.

No zygospores have been found.

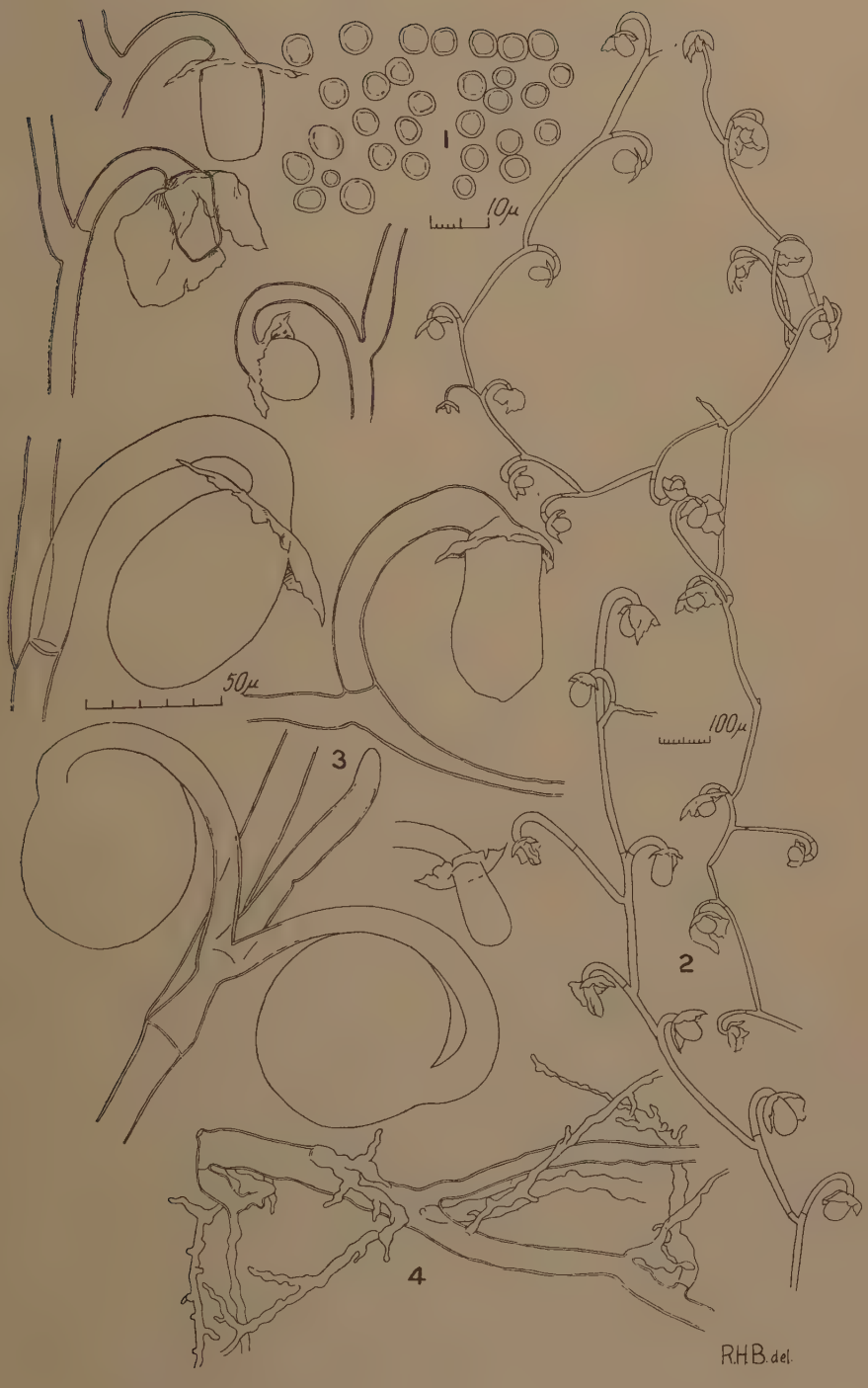
Professor Lendner has kindly provided the diagnosis of this species. It is found in Europe on the excrement of the horse.

III. MOULDS OCCURRING ON COMMERCIALLY DRY CACAO BEANS.

From an economic point of view the most important fungi that infect the interior of cacao-beans are those which persist under conditions of relative dryness. It is unfortunate that, no satisfactory standard of dryness having been fixed, a definition of commercial dryness is impossible. The practice at present adopted by producer and purchaser to judge dryness is by touch, a method of obvious inexactitude and one which has been shown to give results varying from 4.5 to more than 9% of moisture in beans examined by experienced men. Moreover by this means no account can be taken of the relative moisture content of embryo and testa.

Our attempts to ascertain the minimum amount of moisture necessary in any part of a fermented cacao-bean to develop, or maintain, the growth of moulds, have not been concluded. They have been complicated, first by the ever varying moisture content of the bean in its reaction to atmospheric moisture, and again by the number of moulds which occurred. The result of such work as has been accomplished on moisture absorption and its relation to the development of moulds are given in previous papers and it will suffice here to state that an effort to classify cacao

XIII



moulds has singled out the fungus described below as the mould which requires the least amount of moisture for its maintenance. For this reason, and because of the fact that it is capable of penetrating the testa of a bean (see p. 47), it is considered as the most important mould in cacao beans, and as the subject for continued study.

IIIa. Aspergillus glaucus group.

(Plates VII, figs. 1, 2, and 3, VIII, fig. 4, IX, figs 1, 2, and 3).

It is significant that the only species of *Aspergillus* affecting cacao-beans to which reference can be found in literature is a conidial form which Thom and Church (p. 118) consider to be a member of this group. *A. delacroixii* Sacc. and Syd. is reported *in sinubus cotyledonum seminis* in Columbia (Saccardo. 14.1044–45); as causing *maladie du vice propre* in equinoctial America and the West Coast of Africa (*d*) as well as on diseased cacao pods in the Philippine Islands (*e*).

The fungus as it occurs locally in fermented cacao-beans appears either as a deep bluish glaucous (R. XLII. 37″ .d) mass of spore heads (Plate VII, fig. 2), as a pinhard yellow (R. IV. 21.d) collection of perithecia (fig. 3), or as combinations of those colours differing in hue according to the proportion of perithecia scattered among the conidia (fig. 1).

The occurrence of a perithecial stage distinguishes this species from all other forms of *Aspergillus* in cacao-beans. The conidial stage most closely approaches that of A. *fumigatus* in colour and by having a single series of sterigmata; it differs from the latter in sporophores and spores.

The spore heads are at first white, changing from yellow to glaucous blue, and in old growths assuming an olivaceous colour. They are borne on smooth, usually septate, sporophores which increase in width from 6–13μ at the base to 9-19μ at the insertion of the vesicle. The vesicle may be almost spherical and up to 100μ in diameter, or slightly more than twice the diameter of the upper part of the stalk. The simple sterigmata measures from 7–14 x 3-4μ, and occupy practically the whole surface of the vesicle. In culture they tend to proliferate into fantastic forms giving rise to branches which may become sporiferous vesicles, sterile out-growths, or ascogenous hyphae (Plate IX, fig. 2). The conidia are oval to globose, minutely verrucose, 4–7 x 3.5–6μ. The bright yellow perithecia are spherical, from 60 to 120μ in diameter, and arise direct from

(*d*) Delacroix & Maublanc: Maladies des Plantes cultivees dans les Pays Chauds, p. 472.
(*e*) Reinking in Phytopathology V. 9. p. 138.

the recumbent aerial mycelium, they contain a number of irregularly obovate to oval perithecia containing eight spores and measuring 13–17 x 10–12μ. The ascospores are colourless lens-shaped, 3–4.5 x 2.5–3.5μ, with two projecting flanges bordering a median furrow (fig. 3).

The small size of the ascospores precludes the consideration of this fungus as a form of *Eurotium herbariorium* Link. It is probably the organism referred to by Thom and Church (p III) as *A. chevalieri* (Mangin), but efforts to obtain cultures of the latter species for comparison have been unsuccessful, and a critical determination is therefore not yet possible.

IIIb. Penicillium sp.

(Plates VII, figs. 12 and 13, VIII, fig. 11).

Occasionally dry cacao-beans stored under damp conditions become infested by a *Penicillium* which is shown in figure 12 of Plate VII entering the testa by means of the orifice caused by the germinating radicle.

The fungus produces in culture velvety colonies that are slate olive (R. XLVII 29"" i.) in the centre and diminish in tone to a white border. In old cultures of Dox agar it becomes uniformly mummy-brown in colour (R. XV 17′ .m). It bears from two to four sterigmata on the somewhat swollen apices of the branched sporophore; the sterigmata measure 7–15 x 2.5–5μ, and give rise to chains of conidia which may be up to 200μ in length.

The conidia are spherical, smooth, light-green, 2–5μ in diameter in young colonies, and 2–3μ when produced by older growths; in the accompanying figure the spores on the right are from a three months' old culture, the others from a younger growth.